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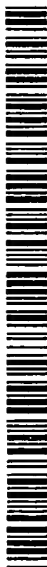International Bureau

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(54) Title: METHODS AND COMPOSITIONS FOR IMMUNOTHERAPY OF B CELL INVOLVEMENT IN PROMOTION OF A DISEASE CONDITION COMPRISING MULTIPLE SCLEROSIS

(57) Abstract: Methods and compositions are provided for reducing a pro-MS immune response by administering to an individual a composition comprising an affinity ligand which binds to B cell determinant, and which is administered in an amount effective to reduce B cells.

METHODS AND COMPOSITIONS FOR IMMUNOTHERAPY OF B CELL
INVOLVEMENT IN PROMOTION OF A DISEASE CONDITION
COMPRISING MULTIPLE SCLEROSIS

5   FIELD OF THE INVENTION

The present invention is related to novel
compositions for use in immunotherapy of an immune response
that contributes to the pathological processes of a disease
condition comprising multiple sclerosis in humans. More
10   particularly, the present invention is related to the
treating B cells which may be involved in promotion of a
chronic inflammatory process involving central nervous
system tissue.

15   BACKGROUND OF THE INVENTION

Multiple sclerosis ("MS") is a chronic
inflammatory disease of the central nervous system. The
characteristic pathological feature, and still used as the
primary basis for diagnosis of MS, is demyelination of the
20   myelin sheath of neurons in the central nervous system. MS
affects 250,000 to 350,000 in the United States, and
approximately 1 million people worldwide. Typically, MS
begins as a relapsing-remitting disease (RRMS) with periodic
episodes of associated symptoms (e.g. various forms of
25   neuritis). Often RRMS eventually changes to a progressive
course of disease, secondary progressive MS (SPMS),
characterized by more CNS tissue damage which results in
more debilitating symptoms. However, in 10 to 20% of
individuals, the disease initially develops in a progressive
30   form known as primary progressive MS (PPMS).

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There is no clear understanding of the immuno-
pathogenic processes associated with MS; and, to date, their
lacks evidence of a unique immunologic abnormality in
individuals with MS. Because of the incomplete under-

5   standing of the pathogenesis of MS, therapeutic advances
have been slow to emerge. The myelin sheath and oligoden-
drocytes are believed to be main targets of autoreactive T
cells which, when activated and reach the central nervous
system, are thought to secrete proinflammatory cytokines.

10   These cytokines are believed to induce astrocytes and
leukocytes (including by activating microglia and
macrophages) to secrete enzymes which damage myelin, and
result in inflammation, demyelination, and axonal damage in
the central nervous system characteristic in MS.

15   Thus, studies have implicated a cell mediated
immune response, T cells recognizing epitopes of myelin
basic protein (MBP), in the pathogenesis of MS. However,
whether the principal effector cells are T cells, or
macrophages, or both T cells and macrophages, is unknown.

20   As shown in Table 1, currently therapies against MS are
either directed to T cells, or are directed to suppressing
the inflammation (e.g., by suppressing macrophages or
microglial cells) that accompanies demyelination.
Whether the humoral immune response is involved in the

25   chronic inflammatory process underlying the pathology of MS
is unproven. For example, anti-ganglioside antibodies have
been reported to be elevated in MS patients. However, it is
unknown whether such antibodies are involved in axonal
damage or are a consequence of axonal damage.

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Table 1

Drug	Action
Betaseron	reduces T cells & gamma interferon
beta 1-Alpha	reduces # of circulating T cells
Cladribine	reduces # of circulating T cells
Copolymer 1	acts as a decoy for T cells
Methotrexate	reduces # of circulating T cells
Myloral	desensitizes T cells to myelin
beta interferon	suppresses microglial cells
methylprednisolone	suppresses macrophages
chlorambucil	suppresses inflammation
cyclophosphamide	suppresses inflammation

Known pathology involving B cells is limited to such diseases as B cell lymphoma. There are numerous approaches

5   and successes in treating B cell lymphoma (cancer cells of B cell origin). Such treatments include administration of immunologically active anti-CD20 antibodies to B cell lymphoma patients; administration of an immunoconjugate comprising mAb Lym-1 coupled to ricin toxin A chain;

10   administration of an immunoconjugate comprising mAb LL2 (anti-CD22) coupled to chemotherapeutic agent; and adminis-tration of an mAb alone, or an immunoconjugate comprising anti-CD19 mAb coupled to a chemotherapeutic agent.

We have discovered that certain soluble antigens,

15   shed from central nervous system (CNS) tissue damaged by an inflam-matory process, are capable of inducing an immune response which promotes one or more of the development, relapse, or progression of MS ("promotion of MS"). We have demonstrated that a similar phenomenon promotes a chronic

20   inflammatory process which enables progression of solid, nonlymphoid tumors.

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Therefore, a need exists for compositions and methods which may be used to therapeutically treat a disease condition selected from the group consisting of MS and a pro-MS immune response, and a pro-MS immune response, by

5   treating B cells in an individual; particularly in an individual who has the disease condition. Further, there is a need to begin disease-modifying therapy early, before the development of irreversible tissue damage and resultant permanent disability.

10

SUMMARY OF THE INVENTION

Accordingly, it is a primary object of the present invention to provide compositions and methods for reducing a pro-MS immune response, wherein the treatment is directed to

15   an individual's immune cells, wherein the immune cells are B lymphocytes, a subpopulation of which may be involved in the promotion of MS.

It is a further object of the present invention to provide methods for treating an individual with a composi-

20   tion for depleting B cells, including to a subpopulation of B cells which are activated by shed antigen released from CNS tissue damaged by an inflammatory process, which B cells may also be found circulating in body fluids selected from the group consisting of peripheral blood, cerebrospinal

25   fluid, and a combination thereof, wherein the depletion of B cells reduces inflammation which causes clinical manifestations associated with progressive MS.

It is also a further object of the present invention to provide methods for reducing a pro-MS immune

30   response as an adjuvant regime in an individual who is

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apparent remission of MS, but whom still has the altered B cell subpopulations comprising a pro-MS immune response, and whom is therefore at risk for a relapse of MS.

5   In another object embodiment, provided is the use of an affinity ligand in the manufacture of a pharmaceutical composition for use in a procedure for depleting nonmalignant B cells associated with a disease condition selected from the group consisting of multiple sclerosis and a pro-MS immune response, and a pro-MS immune response; wherein the
10   affinity ligand selectively contacts and binds to a B cell determinant; wherein the pharmaceutical composition is administered to an individual in an amount effective to deplete B cells; and wherein upon contact and binding to such B cells, the pharmaceutical composition induces or
15   causes B cell depletion. The pharmaceutical composition may further comprise at least one additional component selected from the group consisting of one or more chemotherapeutic agents, an anti-inflammatory agent, a cytolytic agent, a pharmaceutically acceptable carrier, and a combination
20   thereof.

The foregoing objects are based on a discovery of a novel mechanism in which shed antigen, particularly produced and shed by CNS tissue affected by an inflammatory process, is secreted and then induces (activates) a sub-
25   population of B cells, in a humoral immune response, to proliferate and differentiate into plasma cells which produce of anti-shed antigen antibody. Anti-shed antigen antibody can act indirectly and/or directly (via formation of complexes) to exacerbate the ongoing inflammatory
30   process, thereby promoting progression of MS. The objects

of the invention are also achieved by providing methods for depleting B cells, one or more subpopulations of which may be involved in promotion of MS and/or a pro-MS immune response. In one embodiment, administered to an individual

5   is a composition in an amount effective to deplete B cells that may be present infiltrating CNS tissue involved in an inflammatory process; and/or (b) circulating in body fluids, such as peripheral blood and CSF; in an amount effective to deplete B cells. In another embodiment, administered to an

10 individual is a therapeutically effective amount of one or more agents for treating MS (see, e.g., Table 1) in combination or conjunction with the composition for depleting B cells. In these embodiments, the function of the compositions of the present invention is to cause B cell

15 depletion and reduce the exacerbation of inflammation, wherein B cell depletion may include one or more of: blocking of B cell function; functional inactivation of B cells; cytolysis of B cells; inhibiting the proliferation of B cells; inhibiting the production of antibody or inhibiting

20 differentiation of shed antigen-specific B cells; and to cause inactivation or cytolysis of B cells which have been primed or activated by shed antigen. The compositions contact and bind to one or more determinants on B cells and may result in (cause and/or enable) B cell depletion,

25 thereby immunomodulating the immune system to inhibit the MS-promoting function of B cells involved in a pro-MS immune response. Inhibiting the exacerbation of inflammation by depleting B cells involved in a pro-MS immune response may also inhibit a process selected from the group consisting of

further development of MS, progression of MS, relapse of MS,
and a combination thereof.

   The above and other objects, features, and
advantages of the present invention will be apparent in the
5 following Detailed Description of the Invention when read in
conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

 FIG. 1 is a graph showing the effect of depletion on the
10 number of CD19+ cells (B2 cells) of individuals over a
treatment period with a composition in an amount effective
to deplete B cells.

 FIG. 2 is a graph showing the effect of depletion on the
number of CD19+ sTn+ cells (B2 sTn+ cells) of individuals
15 over a treatment period with a composition in an amount
effective to deplete B cells.

 FIG. 3 is a graph showing the effect of depletion on the
number of CD19+ CD21+ sTn+ cells (sTn+ memory/mature B
cells) of individuals over a treatment period with a
20 composition in an amount effective to deplete B cells.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS
Definitions

   The terms "deplete" and "depletion" are used
25 herein in reference to B cells, and for purposes of the
specification and claims, to mean one or more of: blocking
of B cell function; functional inactivation of B cells;
cytolysis of B cells; inhibiting the proliferation of B
cells; inhibiting the differentiation of B cells to plasma
30 cells; causing a B cell dysfunction which results in a

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therapeutic benefit; inhibiting production of anti-shed
antigen antibody; reduction in the number of B cells;
inactivation of B cells which have been primed or activated
by shed antigen; blocking of one or more functions of B

5   cells which have been primed or activated by shed antigen;
cytolysis of B cells which have been primed or activated by
shed antigen; and reduction in the number of B cells which
have been primed or activated by shed antigen. B cell
depletion may be a result of one or more mechanisms

10   including, but not limited to, clonal inactivation,
apoptosis, antibody-dependent cellular cytotoxicity,
complement-mediated cytotoxicity, and a signal pathway
mediated inactivation, dysfunction, or cell death.

        The term "composition" is used herein, for

15   purposes of the specification and claims, to mean a
composition (a) comprised of at least one affinity ligand
which selectively (preferentially) binds to at least one
determinant present on nonmalignant B cells (e.g., mature B
cells and memory B cells); and (b) whereupon when

20   administered in an effect amount to deplete B cells, upon
contact and binding to such B cells, directly or indirectly
results in (causes and/or enables) B cell depletion,
particularly of shed antigen-specific B cells that may be
involved in one or more of a pro-MS immune response, and

25   promotion of progression of MS. Treatment with the
composition may result in a beneficial function. Such a
beneficial function may include, but is not limited to, one
or more of: reduction of the pro-MS immune response by one
or more processes selected from the group consisting of

30   inhibiting the proliferation of B cells which may be

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involved, or may be recruited to be involved, in a pro-MS
immune response, inhibiting production of the anti-shed
antigen antibody (e.g., by reducing the number of B cells
that can be induced to differentiate into anti-shed antigen
5 antibody-secreting plasma cells), reducing the relative
number (e.g., causing or enabling cytolysis) of B cells
which have been primed or activated by shed antigen, and a
combination thereof. By reducing a pro-MS immune response,
the beneficial function may also comprise a reduction in (by
10 inhibiting exacerbation of) the inflammatory process under-
lying clinical manifestations of MS. As an illustrative,
but non-limiting, example, an anti-CD20 mAb, or an anti-Lym-
1 mAb, or an anti-CD19 mAb, or an anti-CD21 mAb, may
selectively bind to B cells (via CD20, Lym-1, CD19, and
15 CD21, respectively) and facilitate or result in B cell
depletion. A bi-specific antibody mAb, anti CD3-CD19 mAb,
may bind to T cells (via CD3) and B cells (CD19) to mediate
T cell-B cell interactions that may facilitate B cell
depletion. Thus, in one embodiment, the present invention
20 provides for the use of at least one affinity ligand in the
manufacture of a pharmaceutical composition comprising a
composition for a procedure for depleting nonmalignant B
cells that may be involved in one or more of a pro-MS immune
response, and promotion of progression MS; wherein the at
25 least one affinity ligand selectively contacts and binds to
a B cell determinant; wherein the B cells may be selected
from the group consisting of mature B cells and memory B
cells, B1 cells, B cell subpopulations of altered amount in
MS (see, e.g., Table 2), and a combination thereof; where-
30 upon contact and binding to such B cells, the composition

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results in B cell depletion; and wherein the procedure
comprises administering to an individual the composition in
an amount effective to deplete B cells. Administering the
composition to an individual may be by a mode which

5   includes, but is not limited to, introducing the composition
by a means to access the individual's CNS tissue to be
treated (e.g., regional or "site-directed" treatment),
introducing the composition into peripheral blood, and a
combination thereof. The composition may further comprise

10   at least one additional component selected from the group
consisting of one or more chemotherapeutic agents, an anti-
inflammatory agent, a cytolytic agent, a pharmaceutically
acceptable carrier, and a combination thereof (see, e.g.,
Table 1). In one embodiment, the additional component is

15   administered as a separate (nonconjugated) component of the
composition. In another embodiment, the additional com-
ponent may be coupled to the affinity ligand; wherein the
affinity ligand serves to selectively bind the B cells,
thereby bringing the additional component in contact with or

20   in functional proximity of B cells that may be involved in
the promotion of progression of MS. A cytolytic agent is an
agent that, by interacting directly with such B cells,
causes B cell cytotoxicity. Such cytolytic agents may
include, but are not limited to, a therapeutically effective

25   amount of an agent selected from one or more of the group
consisting of a toxin, a drug, an enzyme, a cytokine, a
radionuclides, a photodynamic agent, and a molecule which
induce apoptosis (e.g., Fas ligand). A toxin may be
selected from a therapeutically effective amount of one or

30   more of the group consisting of ricin A chain, mutant

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Pseudomonas exotoxins, diphtheria toxoid, streptonigrin, boa-mycin, saporin, gelonin, and pokeweed antiviral protein. A drug may include a therapeutically effective amount of a cytotoxic drug selected from one or more of the group con-

5   sisting of fludarabine, chlorambucil, danazol, daunorubicin, doxorubicin (e.g., in liposomes), cisplatin, bleomycin, melphalan, mitomycin-C, and methotrexate. Due to the sensi-tivity of B cells to radiation, radionuclides may include, but are not limited to, one or more radiometals such as

10   yttrium which emits a high energy beta particle, and I^{125} that emits Auger electrons, that may be absorbed by adjacent B cells. A photodynamic agent may include a therapeutically effective amount of a porphyrin or their derivatives. In addition to the anti-inflammatory agents listed in Table 1,

15   other anti-inflammatory agents are known to those skilled in the art to include COX-2 inhibitors (e.g., rofecxib, and celecoxib), aspirin, ibuprofen, naproxen, and the like. The methods for coupling ligands or targeting molecules with therapeutic agents are well known to those skilled in the

20   art. Often such methods utilize one of several available heterobifunctional reagents used for coupling or linking molecules. Also, the additional component may comprise a pharmaceutically acceptable carrier for facilitating infusion into the bloodstream, CSF, or regional access to

25   the CNS tissue affected by an inflammatory process. Pharmaceutically acceptable carriers are known to those skilled in the art to include buffered saline solutions, buffered carbohydrate solutions, liposomes, sterile water, and the like.

The term "determinant" with reference to B cells, is used herein, for purposes of the specification and claims, to mean a molecule which is preferentially expressed by B cells, or one or more subpopulations thereof which include

5   memory B cells and mature B cells, and one or more B cell subpopulations of altered amounts in MS (see, e.g., Table 2); wherein the molecule is involved in and responsible for selective binding to an affinity ligand having binding specificity and avidity for the determinant. Cell-associated

10 determinants may include, but are not limited to, molecules, receptors, components, or surface immunoglobulin, present on the surface of the cell membrane. "Preferentially expressed" is used herein to mean that the cell-associated determinant is expressed on a substantial number (approximately 40% or

15 greater) of the B cells, or of a subpopulation thereof, which are targeted by the composition. In a preferred embodiment, the determinant is primarily expressed on B cells, with little or no expression of the determinant (as relative to the number of cells expressing the determinant

20 or to the level of expression as compared to B cells) by other subpopulations of immune cells (with the possible exception of dendritic cells; e.g., CD21) contained within a region to which the composition is intended to be targeted. In a preferred embodiment, the determinant is selected from

25 the group consisting of CD19, CD20, CD21, CD22, Lym-1, CDIM, sIg having binding specificity for shed antigen, and a combination thereof.

The term "B cells" is used herein, for purposes of the specification and claims, and in reference to treating a

30 disease condition selected from the group consisting of MS

and a pro-MS immune response, and a pro-MS immune response, to mean mammalian (and preferably human) nonmalignant B cells. As known to those skilled in the art, malignant B cells refers to cancer cells of B cell origin, such as B

5   cell lymphomas, and B cell leukemias. Thus, the term "B cells", as used herein in reference to treating B cells and in treating the disease condition, specifically excludes B cell lymphomas, B cell leukemias, and cancer cells of B cell origin. In that regard, nonmalignant B cells are inclusive

10   of one or more of memory B cells and mature B cells; and B cell subpopulations of altered amounts in MS (e.g., see Table 2) which are involved in one or more of a pro-MS immune response, and promotion of progression of MS, as will be more apparent from the following embodiments.

15   The term "affinity ligand" is used herein, for purposes of the specification and claims, to mean a molecule which has binding specificity and avidity for a determinant associated with B cells that may be present in the area of CNS tissue which is affected by a local inflammatory process

20   (and which produces shed antigen), and/or that may be circu-lating in body fluids such as peripheral blood. In general, affinity ligands are known to those skilled in the art to include, but are not limited to, lectins (or fragments or derivatives thereof which retain specific binding activity),

25   monoclonal antibodies ("mAb", including chimeric or genetically modified monoclonal antibodies which may be preferable for administration to humans), peptides, and aptamers. The term "monoclonal antibody" is also used herein, for purposes of the specification and claims, to

30   include immunoreactive fragments or immunoreactive deriva-

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tives (e.g., peptides) derived from a mAb molecule, which
retain all or a portion of the binding function of the whole
mAb molecule. Such immunoreactive fragments or immunoreac-
tive derivatives are known to those skilled in the art to

5   include F(ab')$_2$, Fab', Fab, Fv, scFV, Fd', Fd, and the like.
Methods for producing the various fragments from mAbs are
well known in the art. For example, F(ab')$_2$ can be produced
by pepsin digestion of the monoclonal antibody, Fab' may be
produced by reducing the disulfide bridges of F(ab')$_2$

10   fragments, and Fab fragments can be produced by papain
digestion of the monoclonal antibody. The construction of
chimeric antibodies is now a straightforward procedure in
which the chimeric antibody is made by joining the murine
variable region to a human constant region. Additionally,

15   "humanized" antibodies may be made by joining the hyper-
variable regions of the murine monoclonal antibody to a
constant region and portions of variable region (light chain
and heavy chain) sequences of human immunoglobulins using
one of several techniques known in the art. Methods for

20   making a chimeric non-human/human mAb in general, and a
chimeric anti-CD20 mAb in particular, are described in
detail in U.S. Patent No. 5,736,137. The chimeric anti-CD20
antibody described in U.S. Patent No. 5,736,137 has been
reported to be therapeutically active on its own; e.g., does

25   not require coupling to a toxin or radioisotope to induce
cytolysis of targeted B cells. Likewise, chimeric anti-CD22
antibody has been previously described in U.S. Patent No.
5,789,554. Likewise, a cross-linking of a B cell by an
anti-CDIM mAb has been reported to induce a cellular

30   response ultimately resulting in cell death (U.S. Patent No.

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5,593,676). In a preferred embodiment, affinity ligands may
include, but are not limited to, a mAb having binding
specificity for one of CD19, CD20, CD21, CD22, CDIM, or Lym-
1. Aptamers can be made against B cell determinants using
5   methods described in U.S. Patent No. 5,789,157.

The term "pro-MS immune response" is used herein,
for purposes of the specification and claims, to mean a
humoral immune response induced against an epitope compris-
ing a terminal alpha 2,6 linked sialic acid (e.g., compris-
10 ing sialyl Tn or sTn which comprises a terminal sialic acid
alpha 2,6 linked to GalNAc; or alternatively, to Gal) of a
shed antigen (glycomolecule), resulting in production of IgG
antibody against the epitope ("anti-α(2,6)NeuAc Ab"), and
complexes comprised of the shed antigen comprising the
15 epitope complexed to anti-α(2,6) NeuAc Ab; wherein the shed
antigen is released or produced particularly in relation to
CNS tissue damage characteristic of MS during the a chronic
inflammatory disease process characteristic of inflammatory
forms of MS (e.g., secondary progressive MS). In a prefer-
20 red embodiment, the resultant complexes bind to and induce
Fc receptor-expressing cells (e.g., one or more cell types
selected from the group consisting of granulocytes, macro-
phages, microglia, activated mast cells, astrocytes, oligo-
dendrocytes) which results in the release of inflammatory
25 mediators (e.g., cytokines and/or tissue degradative
enzymes) which may exacerbate the existing inflammatory
process and thereby promote (contribute to) CNS tissue
damage characteristic of MS (e.g., demyelination and plaques
characteristic of MS). A similar immune response, a pro-
30 tumor immune response, and it's ability to promote

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inflammation and tissue degradation has been previously
described by the present inventors. In a preferred
embodiment, the anti-α(2,6) NeuAc Ab is induced by a shed
antigen comprising glycolipid; and in a more preferred

5   embodiment, glycolipid selected from one or more of the·
alpha series of gangliosides (e.g., GD1α, GT1aα, GQ1bα,
derivatives thereof which contain one or more additional
terminal sialic acids alpha 2,6 linked to GalNAc, and a
combination thereof).

10   The term "individual" is used herein, for purposes
of the specification and claims, to mean a mammal; and
preferably a human. An individual who is at risk of
developing, or has developed, a pro-MS immune response may
include an individual having a form of MS (e.g., secondary

15   progressive form, or relapsing, remitting form); or an
individual who is in apparent remission of MS, but whom
harbors cellular markers characteristic of a pro-MS immune
response (see Table 2) and therefore inherent-ly carries a
risk of relapse or recurrence. The method and compositions

20   according to the present invention are intended for use to
deplete B cells localized in inflammatory infiltrates in
the region of CNS tissue damaged or affected by an inflam-
matory process, and/or circulating in body fluids selected
from the group consisting of peripheral blood, CSF, and a

25   combination thereof, in individuals at risk for developing,
or who have developed, a pro-MS immune response

             The present invention relates to a discovery that
in a pro-MS immune response, a significant number of

30   activated B cells (memory B cells and mature B cells) may be

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retained locally as cells which infiltrate CNS tissue
damaged or affected by an inflammatory process, and/or may
be cells circulating in body fluids selected from the group
consisting of peripheral blood, CSF, and a combination

5   thereof. According to one embodiment of a method for
depleting circulating nonmalignant B cells in a disease
process selected from the group consisting of MS and a pro-
MS immune response, and a pro-MS immune response (also
described herein as a method for reducing a pro-MS immune

10   response) according to the present invention, administered
to an individual is a composition in an amount effective to
deplete B cells. The composition may be administered by a
mode which facilitates infusion into an access which is
regional to the CNS tissue damaged or affected by an inflam-

15   matory response (e.g., by a mode to facilitate delivery of
the composition to demyelinating CNS tissue or plaques
charac-teristic of MS), thereby delivering the composition
in a site-directed manner. The composition may be adminis-
tered by itself, or as part of a regimen of chemotherapeutic

20   agents. In another embodiment of a method for reducing a
pro-MS immune response according to the present invention,
administered to an individual is the composition by a mode
which facilitates infusion into peripheral blood (e.g.,
intravenously) and/or a vascular access (including

25   lymphatics) of the individual's CNS tissue, wherein the
composition is administered in an amount effective to
deplete B cells. The composition may be administered by
itself, or as part of a regimen of chemotherapeutic agents.

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For purposes of the description, the methods and compositions of the present invention will be illustrated in the following examples.

5   **EXAMPLE 1**

This embodiment provides evidence of the B cell involvement in a disease condition selected from the group consisting of MS and a pro-MS immune response, and a pro-MS immune response. We have discovered that there may exist

10   altered phenotype in one or more B cell subpopulations, wherein the phenotype is altered because the relative amounts of these one or more B cell subpopulations that differ in individuals having disease condition selected from the group consisting of MS and a pro-MS immune response, as

15   compared to those values in healthy controls or in individuals having inflammatory diseases other than MS.

For detecting the alterations in the one or more B cell subpopulations in a clinical sample, mononuclear cells were isolated from the clinical sample using a density gradient

20   medium and by density gradient centrifugation. Aliquots, each of approximately 1 million cells, were treated in one of several different ways.

Preferred staining combinations

Memory B cells and sTn+ memory B cells

25   In determining a B cell subpopulation comprising memory B cells, an aliquot of cells was double-stained using anti-CD19 antibody (IgG1 mAb) labeled with Pe-Cy5 (phycoerythrin-Cy5), and an anti-CD21 antibody (IgG1 mAb) labeled with FITC (fluorescein isothiocyanate).

30

For detecting sTn+ memory B cells, an aliquot of cells was triple-stained wherein the cells were first mixed and incubated with anti-sTn antibody (IgG1 mAb), and then washed; followed by mixing and incubating with a secondary rabbit

5   anti-mouse IgG antibody labeled with Pe (phycoerythrin), and then washed; followed by a double-staining with anti-CD19 antibody labeled with Pe-Cy5, and an anti-CD21 antibody labeled with FITC, and then washed. Additionally, by gating on the appropriate parameters, a lymphocyte subpopulation

10   comprising overall B cells (CD19+ cells) may be determined. By gating on the appropriate parameters, a lymphocyte subpopulation comprising sTn+ B cells (CD19+ sTn+ cells) may be determined.

B cells and sTn+ B cells

15   In determining a lymphocyte subpopulation comprising overall B cells, an aliquot of cells was double-stained using essentially the same protocol as summarized above, except that the antibodies mixed with the cells of the this aliquot were anti-CD19 antibody (IgG1 mAb) labeled with Pe-Cy5.

20   Alternatively, for detecting sTn+ B cells, the cells were first mixed and incubated with anti-sTn antibody (IgG1 mAb), and then washed; followed by mixing and incubating with a secondary rabbit anti-mouse IgG antibody labeled with Pe, and then washed; followed by staining with anti-CD19

25   antibody labeled with Pe-Cy5, and then washed. This alternative staining protocol, with the appropriate gating, allows for determination of both overall B cells (CD19+ cells) and sTn+ B cells (CD19+ sTn+ cells).

30

sTn+ B1 cells

In determining a lymphocyte subpopulation comprising sTn+ B1
cells, an aliquot of cells was triple-stained, wherein the
cells were first mixed and incubated with anti-sTn antibody
5   (IgG1 mAb), and then washed; followed by mixing and incubat-
ing with a secondary rabbit anti-mouse IgG antibody labeled
with Pe, and then washed; followed by a double-staining with
anti-CD19 antibody labeled with Pe-Cy5, and an anti-CD5
antibody labeled with FITC, and then washed. Additionally,
10   by gating on the appropriate parameters, a lymphocyte
subpopulation comprising overall B cells (CD19+ cells) may
be determined. Also by gating on the appropriate parameters,
overall B1 cells (CD19+ CD5+ cells) may be determined.

15   A number of commercially available flow cytometers
can be used as the instrument on which is performed the
method of the present invention. Desirably, the flow
cytometer has a single laser source; and in a preferred
embodiment, the single laser source is an argon laser tuned
20   at 488 nanometers (nm). Additionally, the flow cytometer is
operatively connected to appropriate operating software and
data management systems. Using these methods, quantitated
were the relative numbers of one or more B cell subpopula-
tions in clinical samples obtained from individuals having
25   MS and/or a pro-MS immune response; and, as shown in Table
2, the results were compared to the relative numbers of the
same types of B cell subpopulations determined in clinical
samples obtained from apparently healthy individuals
("Baseline control") and determined in clinical samples
30   obtained from individuals having solid, nonlymphoid tumors

and/or a pro-tumor immune response. In this illustration, the clinical samples comprised peripheral blood obtained by venipuncture into blood collection tubes, wherein peripheral blood mononuclear cells were isolated and then analyzed; and

5   the quantitations were performed using flow cytometric methods by the techniques disclosed herein. Light scatter was used as a parameter to gate on primarily lymphocytes based on the size, granularity and cell volume of lymphocytes. In addition to gating for light scatter, each

10   sample undergoing the staining process was gated for respective fluorescence emission(s). For example, when memory B cells were quantitated by double-staining (e.g., for CD19 and CD21), the analysis was gated on those cells positive for CD19 expression as determined by detection of

15   Pe-Cy5 fluorescent emission. CD19 positive lymphocytes were considered to represent the relative overall population of B cells in the clinical sample analyzed. CD19 positive lymphocytes were then gated for those cells also positive for CD21 expression as determined by detection of FITC

20   fluorescent emission. Lymphocytes double stained for both CD19 and CD21 were considered to represent memory B cells. Such CD19+ CD21+ B cells were then expressed as a percentage of overall B cells by using the formula:

25   (the relative number of CD19+ CD21+ cells/ relative number of CD19+ cells) X 100.

         A similar procedure was also used to detect and quantitate a sTn+ B cell subpopulation (e.g., CD19+ sTn+ B

30   cells). Such CD19+ sTn+ B cells were expressed as a percentage of overall B cells by using the formula:

(the relative number of CD19+ sTn+ cells/ relative number of CD19+ cells) X 100.

5 A similar procedure was also used to detect and quantitate a sTn+ memory B cell subpopulation (e.g., CD19+ CD21+ sTn+ B cells). Such CD19+ CD21+ sTn+ B cells were expressed as a percentage of memory B cells by using the formula:

10 (the relative number of CD19+ CD21+ sTn+ cells/ relative number of CD19+ CD21+ cells) X 100.

A similar procedure was also used to detect and quantitate a B1 cell subpopulation (e.g., CD19+ CD5+ B

15 cells). Such CD19+ CD5+ B cells were expressed as a percentage of overall B cells by using the formula:

(the relative number of CD19+ CD5+ cells/ relative number of CD19+ cells) X 100.

20

A similar procedure was also used to detect and quantitate an sTn+ B1 cell subpopulation (e.g., CD19+ CD5+ sTn+ cells). Such CD19+ CD5+ sTn+ B cells were expressed as a percentage of overall B1 cells by using the formula:

25

(the relative number of CD19+ CD5+ sTn+ cells/ relative number of CD19+ CD5+ cells) X 100.

In Table 2, for most B cell subpopulation determinations

30 from apparently healthy individuals (Table 2, "Baseline control") and for most determinations from individuals having solid, non-lymphoid tumors and/or a pro-tumor immune response (Table 2, "Tumor/PTIR"), the relative number is

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expressed as the mean ± the standard error of the mean.
SPMS/PMSIR represents B cell subpopulation determinations
from an individual with secondary progressive MS (SPMS) and
with evidences of a pro-MS immune response (PMSIR) as
5   determined by immunoassays for plasma (anti-shed antigen
antibody, shed antigen, and complexes of anti-shed antigen
antibody + shed antigen).

Table 2

lymphocyte subpopulation	Baseline control	Tumor/PTIR	SPMS/ PMSIR	RRMS/ PMSIR
CD19+	12.7 ± 3.6	2.7 ± 0.5	5.9	13.3
CD19+ sTn+	1.3 ± 0.4	5.9 ± 1.5	18.2	1.6
CD19+ CD21+	1.1 ± 0.7	48.8 ± 5.3	0 .7	3.3
CD19+CD21+sTn+	29.7 ± 5.0	16.1 ± 3.7	42.9	26.0
CD19+ CD5+	1.8	2.4	2.2	-
CD19+CD5+sTn+	0.3	29.0	17.3	-

10

Table 2 illustrates that one or more B cell
subpopulations are altered in relative amount in an
individual having MS and a pro-MS immune response;
particularly in the most inflammatory and aggressive form,
15   SPMS/PMSIR. Note that there is a significant decrease in
the relative number of overall B cells (CD19+ cells) in an
individual having secondary progressive MS and pro-MS immune
response (SPMS/ PMSIR) as compared to values in apparently
healthy individuals (Baseline control). There is a signi-
20   ficant increase in the relative number of sTn+ B cells
(CD19+ sTn+ cells) in an individual having SPMS/PMSIR as
compared to values for the baseline control. While no
significant difference was noted in an overall memory B cell
population (CD19+ CD21+ cells), there is a significant

increase in the relative number of sTn+ memory B cells

(CD19+ CD21+ sTn+ cells) in an individual having SPMS/PMSIR

as compared to values for the baseline control. While no

significant difference was noted in an overall memory B1

5   cell population (CD19+ CD5+ cells), there is a significant

increase in the relative number of sTn+ B1 cells (CD19+ CD5+

sTn+ cells) in an individual having SPMS/PMSIR as compared

to values in the baseline control. Such alterations in the

relative amounts of one or more B cell subpopulations was

10   not observed in the less inflammatory form of MS comprising

primary progressive MS.

                              EXAMPLE 2

             We have discovered that another indication of a

15   pro-MS immune response is the presence in peripheral blood

(e.g., as detected in serum or plasma), and additional

evidence of B cell involvement in the promotion of MS, of

complexes comprising shed antigen complexed to anti-shed

antigen antibody. As an illustration, an enzyme-linked

20   immunosorbent assay (ELISA) was performed. A polystyrene

microtiter plate was coated with a film comprising an

affinity ligand having binding specificity for a terminal

2,6 linked sialic acid. In this illustration, a mouse

monoclonal antibody having such binding specificity was used

25   to coat the wells at a concentration of 10 µg/ml. After

blocking the wells with a blocking solution, the wells

coated with the affinity ligand were then used to detect the

presence of immune complexes in human plasma samples

(diluted 1:50 in buffer) from:

                                24

(1) individuals with no apparent disease or pathology ("Control");

(2) individuals having solid, nonlymphoid tumor ("Cancer"); and

5   (3) individuals having RRMS or SPMS, and a pro-MS immune response ("MS/PMSIR").

The detector molecule comprised anti-human IgG labeled with peroxidase for complexed antibody of the IgG class, and anti-human IgM labeled with peroxidase for detecting

10 complexed antibody of the IgM class. At the appropriate point in the immunoassay procedure, substrate comprising tetramethyl benzidine was added to determine peroxidase activity. The presence of immune complexes was detected by the yellow color (after addition of the sulfuric acid

15 containing stopping reagent), and the color was quantitated at 450 nanometers using a plate reading spectrophotometer. Table 3 illustrates the amount of complexes (circulating complexes with complexed IgG, "CC IgG"; circulating complexes with complexed IgM, "CC IgM") detected as measured

20 by absorbance at 450 nm and expressed as the mean absorbance $\pm$ standard error of the mean (Mean $\pm$ s.e.m.).

Table 3

plasma source	CC IgM Mean $\pm$ s.e.m.	CC IgG Mean $\pm$ s.e.m.
Control	0.37 $\pm$ 0.05	0.41 $\pm$ 0.04
Cancer	0.80 $\pm$ 0.33	0.68 $\pm$ 0.12
MS/PMSIR	0.11 $\pm$ 0.05	1.44 $\pm$ 0.19

25 As shown in Table 3, individuals having RRMS and a pro-MS immune response or SPMS and a pro-MS immune response

25

("MS/PMSIR") have a significant increase in complexes comprised of shed antigen complexed to anti-shed antigen IgG antibody. Such a high concentration of this type of immune complex is an indication of a pro-MS immune response; and of

5   B cell involvement in promotion of MS, wherein such complexes can activate immune effector cells to promote or exacerbate an inflammatory process and tissue degradation.

                              EXAMPLE 3

10   The findings in an individual of one or more B cell subpopulations which are altered in individuals having a pro-MS immune response are indicative of the involvement of one or more subpopulation of B cells in promotion of progression of MS. In such an individual, the B cells may

15   be found infiltrating CNS tissue undergoing demyelination due to an immune reaction and/or an inflammatory response, and/or may be found in body fluids selected from the group consisting of peripheral blood, CSF, and a combination thereof. In method of reducing a pro-MS immune response

20   according to the present invention, administered to an individual is a composition in an amount effective for depleting B cells present in one or more of (a) CNS tissue undergoing demyelination due to an immune reaction and/or an inflammatory response; (b) in body fluids such as peripheral

25   blood. The administered composition subsequently comes in contact with such B cells, and may result in one or more therapeutic functions which may include, but is not limited to, B cell depletion; inhibition of proliferation of B cells involved in a pro-MS immune response; inhibition of activa-

30   tion of B cells by shed antigen; inhibition of differentia-

                                    26

tion of shed antigen-activated B cells into plasma cells capable of secreting anti-shed antigen antibody; causing or enabling inactivation and/or cytolysis of memory B cells which may include a subpopulation of memory B cells that

5 have been primed or activated by shed antigen; and causing or enabling inactivation and/or cytolysis of the one or more B cell subpopulations found in an altered amount (see, e.g., Table 2). In another embodiment, a method for inhibiting the chronic inflammatory process associated with MS and a

10 pro-MS immune response comprises administering the composition in an effective amount to deplete B cells, wherein the composition contacts and binds to one or more determinants on B cells to cause or enable B cell depletion, thereby inhibiting the involvement of B cells in contri-

15 buting to the chronic inflammatory process which promotes a progression of MS. The nerves of the central nervous system which undergo demyelination in MS are often proximal to one or more accesses (e.g., blood or lymphatic or spaces (subarachnoid space)) that feed or extend through the CNS

20 tissue. Thus, to deplete B cells that infiltrate CNS tissues, administered is the composition in one or more accesses in a site-directed method of delivery, wherein the composition is administered in an amount effective to deplete B cells. In one example, it is known that some

25 drugs (e.g., baclofen) are administered intrathecally by bolus injection or by infusions administered by a program- mable pump implanted in the lumbar subarachnoid space. Using standard methods for site-directed delivery, as known to those skilled in the art, the composition may be infused

30 into one or more accesses that directly supplies CNS tissue

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undergoing demyelination ("affected CNS tissue"). The
delivered composition may then concentrate primarily in the
affected CNS tissue; and more specifically to the B cells
present, by the binding between the affinity ligand and its
5   target determinant on the B cells. Site-directed delivery
of the composition does not rule out that a portion of the
composition may gain access to peripheral blood, and any
benefit associated therewith. Likewise, infusion
intravenously may result in the composition reaching B cells
10   infiltrating the affected CNS tissue. This is due, in part,
to the increased blood-brain barrier permeability that is
often observed in individuals having early or late stage
development of a chronic progressive form of MS, and as
particularly localized in demyelinated plaques. The
15   composition may be administered by itself, or in conjunction
with as additional component as previously described herein
in more detail. Also, the composition may further comprise
a pharmaceutically acceptable carrier for facilitating
infusion.

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EXAMPLE 4

      In this example, illustrated is an embodiment of
reducing a pro-MS immune response according to the present
invention, wherein the composition is administered parenter-
25   ally in an amount effective to deplete B cells. The term
"parenterally" includes administration intravenously, intra-
muscularly, subcutaneously, rectally, vaginally, or intra-
peritoneally. The most preferred parenteral administration
is intravenous administration. As will be apparent to one
30   skilled in the art, an amount effective to deplete B cells,

and whether repeated dosages may be warranted, will depend

on such factors as the stage of development of the inflam-

matory process of MS and/or a pro-MS immune response, over-

all health of the individual to be treated, other treatments

5   which the individual may be undergoing, and pharmacokinetic

properties of the type of the composition being used. For

example, for a composition comprising a chimeric anti-CD20

mAb, an effective dose may range from about 0.01 mg/kg of

body weight to about 40 mg/kg of body weight. However, as

10   apparent to one skilled in the art, and in the discretion of

a medical practitioner, a treatment may be warranted with a

dosage falling inside or outside of this illustrative range.

In an illustration of parenteral administration, a composi-

tion comprising a chimeric anti-CD20 mAb may be administered

15   by intravenous infusion in an effective amount to deplete B

cells. The dosage or regimen (multiple doses) may be deter-

mined by monitoring one or more of changes in the status of

MS disease, and measurable parameters of efficacy of the

treatment. Various measurable parameters may include, but

20   are not limited to, the counts or relative numbers of peri-

pheral blood B cell subpopulations (e.g., one or more sTn+ B

cell subpopulations, and other B cell subpopulations; see,

e.g., Table 2), the CD4/CD8 ratio of peripheral blood

lymphocytes, serum or plasma concentration of complexes

25   comprising shed antigen complexed to anti-shed antigen IgG

antibody, and imaging of the affected CNS tissue before and

after treatment.

        To illustrate the method of depleting B cells

according to the present invention, administered to

30   individuals having altered B cell subpopulations (see Table

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2) was a composition comprising a chimeric anti-CD20 mAb administered by intravenous infusion in an amount effective to deplete B cells. Each individual received 200 mg of the composition, and then received two additional infusions

5   approximately every four weeks. Thus, three complete infusions were administered: the initial treatment (week 0), one at week 4, and one at week 8. Generally, the first infusion was at an initial rate of about 50 mg/hour; however, additional infusions were administered at a faster

10   rate which was dependent on how the individual tolerated infusion, the treating physician's judgment, drug manufacturer's instructions, and lack of side effects. As shown in FIGs 1-3, treatment according to the present invention of 3 individuals (■,▲,▼)with the composition

15   resulted in a depletion in overall B cells (CD19+ cells; FIG. 1), and also normalization of the amounts of B cells comprising B cell subpopulations altered in amount (FIG. 2, CD19+ sTn+ cells; FIG. 3, CD19+ CD21+ sTn+ cells) to within a range observed in apparently healthy individuals. Such an

20   observed effect of the composition on depletion of B cells have resulted in a clinical benefit to treated individuals.

     The foregoing description of the specific embodiments of the present invention have been described in

25   detail for purposes of illustration. In view of the descriptions and illustrations, others skilled in the art can, by applying, current knowledge, readily modify and/or adapt the present invention for various applications without departing from the basic concept, and therefore such

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modifications and/or adaptations are intended to be within

the meaning and scope of the appended claims.

What is claimed is:

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1. Use of an affinity ligand in the manufacture of a pharmaceutical composition for use in a procedure for depleting nonmalignant B cells associated with a disease condition selected from the group consisting of multiple

5   sclerosis and a pro-MS immune response, and a pro-MS immune response; wherein the affinity ligand selectively contacts and binds to a B cell determinant; wherein the pharmaceutical composition is administered to an individual in an amount effective to deplete B cells; and wherein upon

10   contact and binding to such B cells, the pharmaceutical composition induces or causes B cell depletion.

2. The use according to claim 1, wherein the pharmaceutical composition further comprises at least one additional

15   component selected from the group consisting of one or more chemotherapeutic agents, an anti-inflammatory agent, a cytolytic agent, a pharmaceutically acceptable carrier, and a combination thereof.

20   3.   The use according to claim 1, wherein the nonmalignant B cells are B cells selected from the group consisting of mature B cells and memory B cells, CD19+sTn+ B cells, CD19+CD21+sTn+ B cells, CD19+CD5+sTn+ B cells, and a combination thereof.

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4.   The use according to claim 1, wherein the affinity ligand comprises a chimeric anti-CD20 monoclonal antibody.

5.   The use according to claim 1, wherein the pharmaceutical

30   composition is administered by a mode selected from the group consisting of parenterally, and in a site-directed method in which the pharmaceutical composition is delivered

into an access that directly supplies central nervous tissue
undergoing demyelination.

6.   Use of an affinity ligand in the manufacture of a
5   pharmaceutical composition for use in a method for reducing
a pro-MS immune response in an individual, the method
comprising administering to an individual the pharmaceutical
composition, wherein the pharmaceutical composition
comprises an affinity ligand which selectively binds to a B
10   cell determinant, wherein the B cells targeted by the method
and by the pharmaceutical composition are nonmalignant B
cells, and wherein the pharmaceutical composition is
administered in an amount effective to deplete B cells.

15   7. The use according to claim 1, wherein the nonmalignant B
cells are B cells selected from the group consisting of
mature B cells and memory B cells, CD19+sTn+ B cells,
CD19+CD21+sTn+ B cells, CD19+CD5+sTn+ B cells, and a
combination thereof.
20

8.   The use according to claim 1, wherein the affinity
ligand comprises a chimeric anti-CD20 monoclonal antibody.

9.   The use according to claim 1, wherein the pharmaceutical
25   composition is administered by a mode selected from the
group consisting of parenterally, and in a site-directed
method in which the pharmaceutical composition is delivered
into an access that directly supplies central nervous tissue
undergoing demyelination.
30

10.   The use according to claim 6, wherein the
pharmaceutical composition further comprises an additional
component selected from the group consisting of one or more
chemotherapeutic agents, an anti-inflammatory agent, a
35   cytolytic agent, a pharmaceutically acceptable carrier, and
a combination thereof.

11. Use of an affinity ligand in the manufacture of a
pharmaceutical composition for use in a site-directed method
for reducing a pro-MS immune response in an individual, the
5   method comprising administering to an individual the
pharmaceutical composition, wherein the pharmaceutical
composition comprises an affinity ligand which selectively
binds to a B cell determinant, wherein the B cells targeted
by the method and by the pharmaceutical composition are
10   nonmalignant B cells, which the pharmaceutical composition
is delivered into an access that directly supplies central
nervous tissue undergoing demyelination, and wherein the
pharmaceutical composition is administered in an amount
effective to deplete B cells.

15

12. The use according to claim 11, wherein the nonmalignant
B cells are B cells selected from the group consisting of
mature B cells and memory B cells, CD19+sTn+ B cells,
CD19+CD21+sTn+ B cells, CD19+CD5+sTn+ B cells, and a
20   combination thereof.

13. The use according to claim 11, wherein the affinity
ligand comprises a chimeric anti-CD20 monoclonal antibody.

25   14. The use according to claim 11, wherein the
pharmaceutical composition further comprises an additional
·   component selected from the group consisting of one or more
chemotherapeutic agents, an anti-inflammatory agent, a
cytolytic agent, a pharmaceutically acceptable carrier, and
30   a combination thereof.

15. Use of an affinity ligand in the manufacture of a
pharmaceutical composition for use in a method for reducing
a pro-MS immune response in an individual, the method
35   comprising administering to an individual the pharmaceutical
composition, wherein the pharmaceutical composition

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comprises an affinity ligand which selectively binds to a B
cell determinant, wherein the B cells targeted by the method
and by the composition are nonmalignant B cells, wherein the
pharmaceutical composition is administered intravenously,
5   and wherein the pharmaceutical composition is administered
in an amount effective to deplete B cells.

16. The use according to claim 15, wherein the nonmalignant
B cells are B cells selected from the group consisting of
10   mature B cells and memory B cells, CD19+sTn+ B cells,
CD19+CD21+sTn+ B cells, CD19+CD5+sTn+ B cells, and a
combination thereof.

17. The use according to claim 15, wherein the affinity
15   ligand comprises a chimeric anti-CD20 monoclonal antibody.

18. The use according to claim 15, wherein the
pharmaceutical composition further comprises an additional
component selected from the group consisting of one or more
20   chemotherapeutic agents, an anti-inflammatory agent, a
cytolytic agent, a pharmaceutically acceptable carrier, and
a combination thereof.

19. Use of an affinity ligand in the manufacture of a
25   pharmaceutical composition for use in a method for treating
an individual having a disease condition selected from the
group consisting of multiple sclerosis (MS) and a pro-MS
immune response, and a pro-MS immune response; wherein the
pharmaceutical composition comprises an affinity ligand
30   which selectively binds to a B cell determinant, wherein the
B cells targeted by the method and by the pharmaceutical
composition are nonmalignant B cells, and wherein the
pharmaceutical composition is administered in an amount to
effect a reduction in inflammation underlying clinical
35   manifestations of MS.

20. The use according to claim 19, wherein the nonmalignant B cells are B cells selected from the group consisting of mature B cells and memory B cells, CD19+sTn+ B cells, CD19+CD21+sTn+ B cells, CD19+CD5+sTn+ B cells, and a
5   combination thereof.

21. The use according to claim 19, wherein the affinity ligand comprises a chimeric anti-CD20 monoclonal antibody.

10   22. The use according to claim 19, wherein the pharmaceutical composition further comprises an additional component selected from the group consisting of one or more chemotherapeutic agents, an anti-inflammatory agent, a cytolytic agent, a pharmaceutically acceptable carrier, and
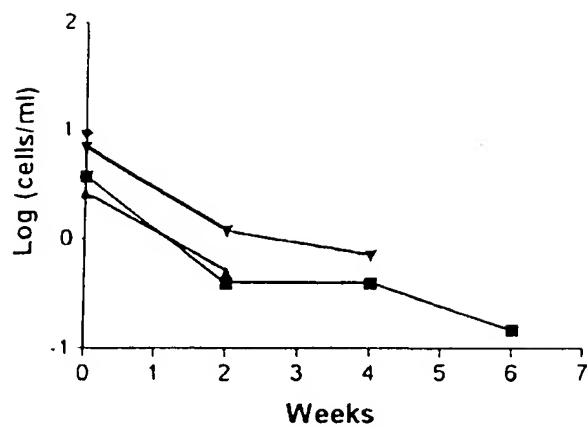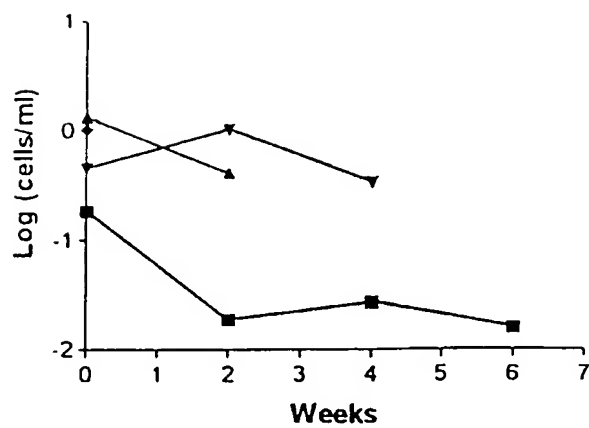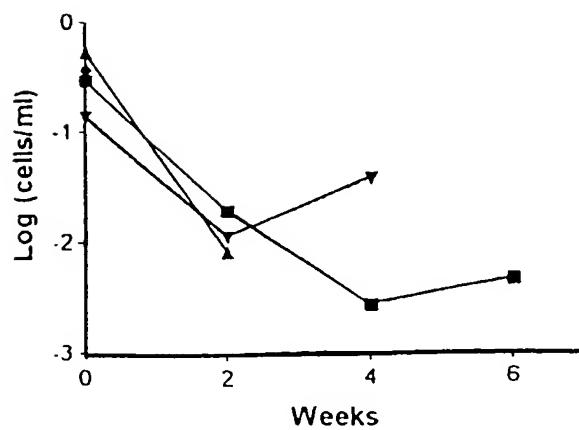15   a combination thereof.

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Fig. 1

Fig. 2

Fig. 3

INTERNATIONAL SEARCH REPORT

A.	**CLASSIFICATION OF SUBJECT MATTER**

IPC(7) : A61K 39/00, 39/395
US CL   : 424/133.1, 144.1, 153.1, 173.1, 801
According to International Patent Classification (IPC) or to both national classification and IPC

B.	**FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. :   424/133.1, 144.1, 153.1, 173.1, 801

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST 2.0, MEDLINE, BIOSIS search terms: cd20, antiCD20, antibod?, pharmaceutical?, author name

C.	**DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,736,137 A (ANDERSON et al.) 07 April 1998, see entire document.	1-22

☐ Further documents are listed in the continuation of Box C.	☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 SEPTEMBER 2000	1 8 OCT 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer RON SCHWADRON
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